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Cancer-Associated Osteoclast Differentiation Takes a Good Look in the miR(NA)ror

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Tumor-bone cell interactions are critical for the development of metastasis-related osteolytic bone destruction. In this issue of *Cancer Cell*, Ell and colleagues show how a discrete miRNA network regulates osteoclastogenesis during breast cancer bone metastasis. A signature of upregulated miRNAs may have diagnostic and therapeutic implications for bone metastases.

Advanced breast cancer commonly metastasizes to bone, where it causes osteolytic bone destruction and associated bone pain and fracture, hypercalcemia, and paralysis due to spinal cord compression. In the bone microenvironment, tumor cells hijack the bone remodeling process, normally orchestrated by osteoclasts, osteoblasts, and osteocytes, to wreak havoc and weaken the bone. Osteoclast differentiation and bone resorption is dependent on macrophage colony-stimulating factor and receptor activator of NF-κB ligand (RANKL) (Boyle et al., 2003). Once in the bone, breast cancer cells release factors that send osteoclasts into overdrive by recruiting preosteoclasts and inducing their differentiation. Osteoclastic bone resorption releases growth factors stored in the bone, such as transforming growth factor β (TGF-β), which in turn drives tumor cell production of factors that further increase osteoclast activity (Weilbaecher et al., 2011). This feed-forward vicious cycle creates a fertile microenvironment for tumor growth in bone to drive the devastating effects of bone destruction and render the tumor incurable.

Therapy for patients with bone metastases attacks the tumor cells as well as the bone microenvironment. Antiresorptive therapy, bisphosphonates (zoledronic acid), and the RANKL antibody (denosumab) are standard-of-care to target osteoclast hyperactivity. These drugs effectively reduce skeletal-related events due to bone metastases but do not cure disease. Further, it is difficult to predict who will develop bone metastases due to lack of broadly applicable biomarkers to better guide long term preventive therapy.

In this issue of *Cancer Cell*, Ell et al. (2013) propose a single approach to treat and predict bone metastases based on microRNA (miRNA). Specifically, they identify a miRNA signature induced by highly metastatic tumor cells that stimulates differentiation of osteoclasts and recruits preosteoclasts to the site of the tumor-bone interface (Figure 1). miRNAs repress gene expression through complementary binding to the "seed sequence" of mRNAs (Bartel, 2009) and are important for osteoclastogenesis (Mizoguchi et al., 2010; Sugatani and Hruska, 2007; Zhang et al., 2012). Here, the authors demonstrate how breast cancer cell invasion in the bone co-opts this normal process to hyperactivate osteoclasts and prime the bone for osteolytic destruction. This represents significant insight into our understanding of the organ-specific function and pathological activity of miRNAs, which could lead to improvements in diagnosis, treatment, and prevention of bone metastases and elucidates a unique aspect of the bone microenvironment to support tumor growth in bone.

To identify miRNAs modulated during osteoclastogenesis, the authors used

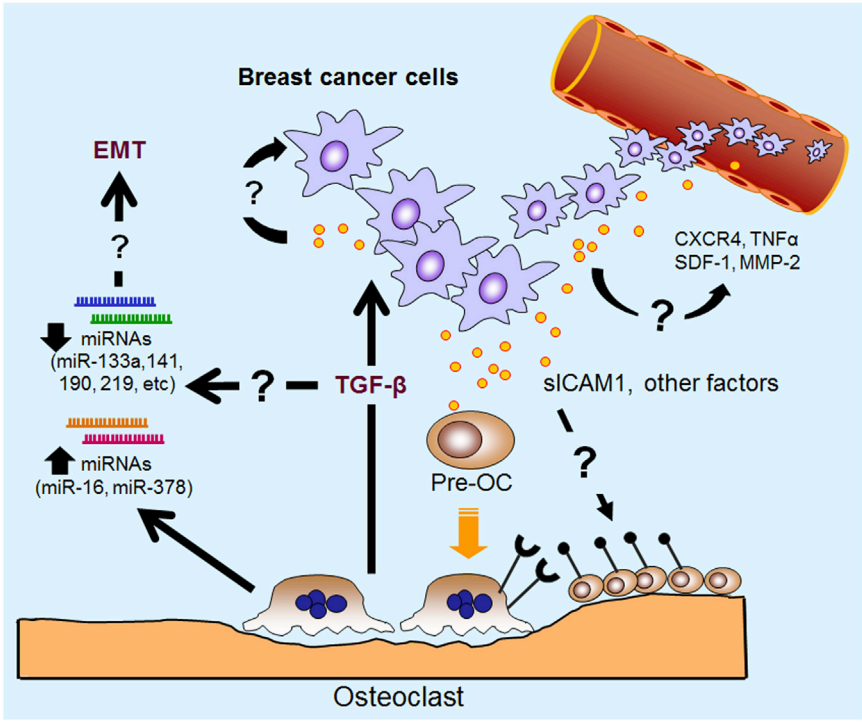

Figure 1. Feed-Forward Vicious Cycle of Bone Metastases Involves sICAM1 and miRNAs
Highly metastatic tumor cells infiltrating the bone microenvironment from the vasculature, secrete soluble forms of ICAM1 (sICAM1, orange balls), which stimulate a distinct miRNA expression signature in osteoclasts to fuel osteoclastogenesis and bone destruction, are shown. miRNA expression correlates with expression of sICAM1 from tumor cells. This increased bone resorption releases TGF-β from the bone matrix. In this setting, TGF-β could potentially act on tumor cells to further increase ICAM1 expression as well as miRNAs that influence epithelial-mesenchymal transition (EMT). sICAM1 may play a significant role in both of these pathways. sICAM1 indirectly influences bone resorption, as demonstrated in Ell et al. (2013). In addition, ICAM1 expressed on osteoblasts acts as a ligand for lymphocyte function-associated antigen-1 (LFA-1) on preosteoclasts (Pre-OC) to stimulate osteoclastogenesis. sICAM1 secreted by tumors may also act in an autocrine fashion on cancer cells. sICAM1 can facilitate tumor adhesion, migration and extravasation by interaction with factors known to influence tumor invasion, such as CXCR4, TNFα, SDF-1, and MMP-2.

conditioned media (CM) from tumor cells (breast and bladder cancer) with high metastatic potential with or without RANKL to compare miRNA expression changes in RAW264.7 osteoclast precursor cells. Forty two upregulated and 45 downregulated miRNAs (>2.2-fold change) were identified across treatment groups. Five miRNAs that were significantly downregulated during osteoclastogenesis in both physiological and pathophysiological conditions (miR-33a, miR-133, miR-141, miR-190, and miR-219) were further analyzed. Ectopic expression of miR133a, miR-141, and miR-219 strongly inhibited osteoclastogenesis. The effect of this miRNA signature on osteoclast activity was validated by the examination of genes known to be important for osteoclast differentiation (*Mitf, Calcr*, *Traf6*, and *Mmp14*).

Remarkably, miR-141 and miR-219 administered systemically led to a significant decrease in the number of osteoclasts in vivo, and these same miRNAs reduced metastatic tumor burden in an experimental breast cancer (MDA-MB-231) bone metastasis model. The therapeutic effect of systemic miRNA treatment in mice with MDA-MB-231 bone metastases was as effective to reduce bone metastases as the standard-of-care, zoledronic acid.

A signature of upregulated miRNAs (miR-16, miR-211, miR-378, and Let-7a) during osteoclast differentiation was also determined. Because these did not significantly impact osteoclastogenesis, the authors thought to investigate this signature as a useful biomarker for osteolytic bone metastasis. miR-16 and miR-378 were consistently increased in serum from mice with highly metastatic breast cancer cells and in serum from patients with breast cancer metastatic to bone as compared to healthy female donors.

Osteoclastogenesis induced by tumor cell CM showed a similar set of miRNA changes as RANKL treatment and led the authors to determine what factor(s) in CM could induce osteoclast activity. Cytokine expression analysis of CM revealed that soluble ICAM1 (sICAM1) was released from highly metastatic tumor cells and enhanced osteoclast activation. In addition, sICAM1 enhanced migration of preosteoclasts in vitro, suggesting it may also play a role in the recruitment of preosteoclasts to the site of bone resorption, though this is yet to be confirmed in vivo. It has also been reported that expression of ICAM1 on osteoblasts could directly bind lymphocyte function-associated antigen-1 (LFA-1) on preosteoclasts to drive osteoclastogenesis (Figure 1) (Tanaka et al., 2000). Neutralizing antibody to ICAM1 has also been shown to reduce osteoclast formation via this mechanism. ICAM could also be involved in the recruitment of tumor cells to the site of bone metastasis directly, as it is known to increase migration and adhesion of MDA-MB-231 cells under inflammatory conditions (Evani et al., 2013).

To investigate the clinical significance of sICAM1 as a tumor-derived factor involved in osteoclastogenesis, the authors measured serum sICAM1 concentrations from healthy donors and patients with either primary (local) breast cancer or breast cancer with bone metastases. Serum sICAM1 concentrations were significantly increased in patients with bone metastases and correlated with an increase in miR-16 and miR-378. In addition, miR-16 exhibited higher specificity than N-terminal telopeptide, the standard marker of bone turnover, in serum samples from patients with bone metastases relative to patients without bone metastases or healthy donors.

This study elegantly shows that a miRNA regulatory network is strongly involved in both physiological and pathological bone remodeling. This is the best description yet of an organ-specific role of miRNA regulation and how dysregulation of miRNAs during bone metastasis correlates with metastatic capacity. These findings also raise important

questions (Figure 1). First, could the miRNAs identified in this study inhibit tumor cells directly? The authors state that these miRNAs do not have any effect on the growth or survival of tumor cells in vitro, but the in vivo possibility remains. Second, does expression of sICAM1 from tumor cells at a distant site prime them to metastasize to bone or do tumor cells require cues from the bone-tumor microenvironment to elicit an increase in ICAM1? Finally, because TGF-β is released in large amounts during cancer-induced bone destruction, could TGF-β directly regulate miRNAs? For example, it can induce miRNAs in MDA-MB-231 breast cancer, which increases metastatic potential and epithelial-mesenchymal transition (EMT) (Taylor et al., 2013), but it also represses miRNAs that result in the same effect (Ding et al., 2013).

Use of the technology described in this study to identify and validate miRNAs as diagnostic and therapeutic tools is powerful. Could these miRNAs identify patients at higher risk for bone metastasis? Could miRNA therapy be as effective as current antiresorptive therapy? The studies in mice predict that the miRNAs will be as effective as zoledronic acid in humans, but it will be important to compare to denosumab, which is about 20% more effective to reduce skeletal-related events in head-to-head trials with zoledronic acid. Will miRNA antiresorptive therapy be associated with potential adverse events such as osteonecrosis of the jaw or atypical fractures? Do the miRNAs have effects on osteoblasts and osteocytes? Are miRNAs dysregulated in other diseases also associated with increased osteoclast activity such as osteoporosis, Paget's disease, or fibrous dysplasia? Answers to these important questions will shed new light into the pathophysiology of bone metastases, the tumor microenvironment, as well as regulation of osteoclastogenesis and have important implications for the diagnosis, prognosis, and treatment of this devastating complication of malignancy.

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